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Within-herd prevalence of intramammary infection caused by *Mycoplasma bovis* and associations between cow udder health, milk yield, and composition

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ABSTRACT

Subclinical mastitis is one of the major health problems in dairy herds due to decreased milk production and reduced milk quality. The aim of this study was to examine the within-herd prevalence of subclinical intramammary infection caused by *Mycoplasma bovis* and to evaluate associations between *M. bovis* and cow daily milk yield, udder health, and milk composition. Individual cow composite milk samples (n = 522) were collected from all lactating dairy cows in 1 Estonian dairy farm in November 2014. Daily milk yield, days in milk, and parity were recorded. Collected milk samples were analyzed for somatic cell count, milk protein, fat, and urea content. The presence of *M. bovis*, *Staphylococcus aureus*, *Streptococcus agalactiae*, and *Streptococcus uberis* in the milk samples was confirmed by quantitative PCR analysis. The within-herd prevalence of *M. bovis* was 17.2% in the study herd. No association was observed between days in milk and parity to the presence of *M. bovis* in milk. According to linear regression analysis, the daily milk yield from cows positive for *M. bovis* was on average 3.0 kg lower compared with cows negative for *M. bovis*. In addition, the presence of *M. bovis* in milk samples was significantly associated with higher somatic cell count and lower fat and urea content compared with milk samples negative for *M. bovis*. In conclusion, subclinical *M. bovis* intramammary infection is associated with decreased milk yield and lower milk quality.

Key words: *Mycoplasma bovis* mastitis, dairy cow, prevalence, milk yield

INTRODUCTION

Mastitis, which can be caused by different udder pathogens, is one of the major concerns in dairy herds because it causes economic losses to the industry due to lower milk production and reduced milk quality (Ruegg, 2012; Hertl et al., 2014). In addition, milk fat and protein concentration have been shown to decrease due to lipolysis and proteolysis in mastitic milk (Larsen et al., 2010; Vidanarachchi et al., 2015; Zhang et al., 2015).

The biggest effect on dairy herd milk quality and production arises from contagious mastitis pathogens such as *Staphylococcus aureus*, as well as *Streptococcus agalactiae* (Reksen et al., 2007; Paradis et al., 2010; Sørensen et al., 2010). *Mycoplasma bovis*, a bacterium lacking a cell wall from genus *Mycoplasma*, mainly causes IMI (Nicholas and Ayling, 2003; Maunsell et al., 2011). *Mycoplasma bovis* is usually classified as a contagious mastitis pathogen (USDA APHIS, 2008; Royster and Wagner, 2015). Transmission between animals occurs mainly at the milking time (Ruegg, 2012). *Mycoplasma bovis* usually causes subclinical or mild clinical IMI, which can progress to chronic mastitis. Severe clinical mastitis outbreaks may also develop (Bushnell, 1984; Pothmann et al., 2015; Ruegg and Erskine, 2015). *Mycoplasma bovis* mastitis is not treatable with antibiotics; therefore, the control strategies of *M. bovis* IMI are to keep the herd uninfected, and to segregate and cull infected cows (Fox et al., 2005; Royster and Wagner, 2015; Nicholas et al., 2016).

Traditionally, *M. bovis* has been identified using culture-based methods, but due to low sensitivity and a long incubation period, molecular diagnostic methods have become preferable during recent years (Dorman et al., 1983; Sachse et al., 2010; Gioia et al., 2016). Detection of *M. bovis* in bulk tank milk samples (**BTMS**) or cow composite milk samples (**CMS**) by using quantitative PCR (**qPCR**) allows an identification of udder pathogens, including *M. bovis*, rapidly, with high

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sensitivity, and without previous isolation of bacteria (Ghadersohi et al., 1997; Ghadersohi et al., 1999; Fox et al., 2005). Identification of infected herds is best made by analyzing BTMS. *Mycoplasma bovis* positive result indicates that the pathogen is introduced to the herd. However, the true within-herd prevalence cannot be estimated with only a positive BTMS result. Further identification of infected cows should be made by analyzing CMS (Fox et al., 2005).

Although mycoplasmas were identified in North America and Europe decades ago, the prevalence of *M. bovis* mastitis is not widely studied (Fox et al., 2005; Fox, 2012). The herd prevalence of *M. bovis* udder infection ranges between 0.9% in Australia and 1.5% in Belgium (Passchyn et al., 2012; Morton et al., 2014). According to a longitudinal study made in Israel, the number of *M. bovis*-positive dairy herds has increased annually from 2008 to 2014 (Lysnyansky et al., 2016). However, the within-herd prevalence of *M. bovis* mastitis is still not widely studied to this day. By knowing the within-herd prevalence and course of the disease, it could be possible to develop functional control programs and predict new outbreaks. In a study by Murai et al. (2014), a within-herd prevalence of *M. bovis* mastitis was 2.8% (n = 1,210). According to the Estonian Animal Recording Centre database, 19.6% of BTMS (n = 112), analyzed with the PCR method, were positive to *M. bovis* in 2013 (Estonian Animal Recording Centre, 2014).

To our knowledge, no studies are available about the associations between *M. bovis* mastitis and cow milk yield or milk composition. Research about this, however, would clarify the importance of *M. bovis* as an udder pathogen and as a cause of production losses.

The first objective of this study was to identify the within-herd prevalence of subclinical IMI caused by *M. bovis* by qPCR method. The second aim was to find associations between subclinical *M. bovis* infection, cow daily milk yield, SCC, and milk composition.

MATERIALS AND METHODS

Characteristics of the Study Herd

The milk samples were collected from one large, loose-housing dairy herd in Estonia in November 2014. The study herd included 611 dairy cows, of which 89% were Estonian Holstein, and 11% were Estonian Red breed cows. Cows were milked twice a day in the 2 × 12 parallel milking parlors. The average 305-d milk yield was 9,916 kg and bulk milk SCC ranged between 259,000 and 358,000 cells/mL in 2014. *Mycoplasma bovis* was previously detected in BTMS and cow CMS of single cows with clinical mastitis by PCR in 2011.

Collection and Analysis of Composite Milk Samples

The CMS of all 525 lactating dairy cows were collected once during the routine milk recording in November 2014. The daily milk yield of each cow was measured by using a calibrated milk meter (Tru-Test Limited, Auckland, New Zealand). Parity and DIM of all lactating dairy cows were recorded.

All milk samples were preserved with bronopol and transported to the milk laboratory of the Estonian Animal Recording Centre in Tartu. In the laboratory, milk fat (%), protein (%), urea (mg/L), and SCC (× 1,000/mL) were analyzed with accredited methods using the automatic analyzer Combifoss 6000 FC (Hillerød, Denmark).

After analysis, 1.5 mL of each milk sample was collected and transported to the Estonian University of Life Sciences. Cows with visible signs of clinical mastitis (n = 3) were excluded from the study, and 2 cows without production data were excluded only from the regression analysis. All milk samples (n = 522) were stored at −18°C for further analysis.

qPCR Analysis of Milk Samples

A commercial qPCR test kit Mastit4B (DNA Diagnostic A/S, Risskov, Denmark) was used for qPCR analysis to detect bacterial DNA directly from the milk samples.

The oligos of the Mastitis 4B are designed to detect DNA of *Staph. aureus*, *Strep. agalactiae*, *Strep. uberis*, and *M. bovis.* After thawing, the milk samples were vortexed and from each sample, 500 μL of milk was used for DNA extraction before PCR analysis according to the instructions (http://dna-diagnostic.com/files/Downloads/Mastit4/Instruction_protocol_M4B_2017.04.26.pdf) from the manufacturer (DNA Diagnostic, Risskov, Denmark). The PCR mixture consisted of 15 μL of the qPCR Master Mix and 5 μL of purified DNA. The real-time PCR instrument thermal cycler Stratagene Mx3005P (Agilent Technologies Inc., Santa Clara, CA) was used for amplification. The amplification conditions were as follows: 95°C for 1 min, 1 cycle; 95°C for 5 s and 60°C for 25 s, 40 cycles. Cycle threshold (**Ct**) values were reported for all samples. For all bacteria identified in the analysis, a Ct value of ≤37.0 was considered a positive result. The assay included controls for the validation of each run including negative DNA extraction controls, internal amplification standard

Table 1. Different combinations of detected bacteria in pathogen-positive milk samples

Pathogen	Number of positive samples (%)
Streptococcus agalactiae	75 (37.5)
Mycoplasma bovis	30 (15.0)
Staphylococcus aureus	14 (7.0)
Streptococcus uberis	3 (1.5)
Mycoplasma bovis, *Streptococcus agalactiae*	39 (19.5)
Streptococcus agalactiae, *Staphylococcus aureus*	17 (8.5)
Mycoplasma bovis, *Staphylococcus aureus*, *Streptococcus agalactiae*	15 (7.5)
Mycoplasma bovis, *Staphylococcus aureus*	6 (3.0)
Streptococcus agalactiae, *Staphylococcus aureus*, *Streptococcus uberis*	1 (0.5)
Total	200 (100)

(positive PCR controls), and nontemplate control. The assay was validated on both bacterial strains and milk samples by DNA Diagnostic. According to the internal validation protocol of the laboratory, the sensitivity and specificity of the test kit Mastitis 4B is 100% when tested directly on a bacterial colony.

Statistical Analyses

A causal diagram was drawn for variables to evaluate their causal associations and identify any confounders. Dependent variables were milk yield (kg), SCC (× 1,000/mL), milk protein, fat percentages, and milk urea content (mg/L). Cow parity and DIM were considered to be confounders according to the causal diagram. A linear regression model was chosen for estimating the associations between udder pathogens and milk yield, SCC, and milk compositions. The distribution of dependent variables was checked graphically. To achieve normal distribution, natural logarithm transformation was used for SCC, the square root was taken from the milk fat content, and an inverse scale was used for milk protein content.

The PCR test results were dichotomized for each bacterium as either presence or absence by using the cut-off values (≤37.0) set by the manufacturer. Associations between the presence of 3 mastitis pathogens (*M. bovis*, *Staph. aureus*, and *Strep. agalactiae*) cow parity, and DIM were assessed using the χ^2 test. Due to low number of qPCR-positive *Strep. uberis* CMS, the cow infection status of that pathogen was not added to the risk factor models. Statistical significance was set at $P \leq 0.05$.

Multivariable models were composed separately for each production indices serving as outcome variables. In multivariable models, cow parity (categorized into 1, 2, and ≥3 lactations) and DIM (categorized as 1–90, 91–200, and ≥201 DIM) were inserted to control for confounding effects. Dichotomized results (yes = 1, no = 0) of *M. bovis*, *Staph. aureus*, and *Strep. agalactiae* were included to assess the association with the outcome variable. Interaction terms were tested for significance to see whether the combined effect of 2 mastitis pathogens differs from the sum of the individual effects of the pathogens tested (Dohoo et al., 2009). Assumptions of the equal variance of the outcome in all levels of predictor variables and normal distribution of the residuals were checked graphically (Dohoo et al., 2009).

The STATA IC 10 (StataCorp, College Station, TX) software was used for statistical analyses.

RESULTS

Descriptive Statistics

Out of 522 cow CMS, 38.3% (n = 200) contained DNA from at least one targeted bacterial species. The DNA of one detected bacterial species was found in 61% (n = 122), 2 species in 31% (n = 62), and 3 species were detected simultaneously in 8% (n = 16) of pathogen-positive milk samples. *Mycoplasma bovis* alone was detected in 15% (n = 30) of pathogen-positive milk samples and in combination with *Strep. agalactiae* in 19.5% (n = 39) of pathogen-positive milk samples. The most prevalent bacterial species was *Strep. agalactiae* alone, presented in 37.5% (n = 75) of pathogen-positive milk samples. The different combinations of udder pathogens in pathogen-positive cow CMS are present in Table 1.

Prevalence of Udder Pathogens

The within-herd prevalence of *M. bovis* was 17.2% (n = 90; 95% CI 14.1–20.8). *Streptococcus agalactiae* had the highest within-herd prevalence of 28.4% (n = 148; 95% CI 24.5–34.2). The within-herd prevalence of *Staph. aureus* was 10.2% (n = 53; 95% CI 7.7–13.1). *Streptococcus uberis* was found only in 5 CMS with prevalence of 1% (95% CI 0.3–2.2).

The presence of *M. bovis* or *Staph. aureus* was not significantly associated with DIM. The probability of detecting *Strep. agalactiae* in milk samples was higher

Table 2. Distribution of detected mastitis pathogens (alone or in combination) in composite milk samples of 520 dairy cows according to lactation stage and parity

Item	Number of cows	*Mycoplasma bovis* positive, no. (%)	*Staphylococcus aureus* positive, no. (%)	*Streptococcus agalactiae* positive, no. (%)
DIM				
0–90	133	23 (17.3)	15 (11.3)	25 (18.8)
91–200	170	35 (20.6)	15 (8.8)	53 (31.2)
≥201	217	32 (14.7)	23 (10.6)	70 (32.2)*
Parity				
1 Lactation	220	29 (13.2)	18 (8.2)	58 (26.4)
2 Lactations	159	30 (18.9)	13 (8.2)	49 (30.8)
≥3 Lactations	141	31 (22.0)	22 (15.6)*	41 (29.1)

*Statistically significant in χ^2 test ($P < 0.05$) by DIM or lactation number.

($P < 0.05$) in dairy cows in late lactation stage (>201 DIM) compared with the first 3 mo of lactation. There was a significantly higher risk of detecting *Staph. aureus* in milk samples from older cows (≥3 lactations) compared with ≤2 lactation dairy cows (Table 2).

Associations Between Detected Mastitis Pathogens and Milk Yield, Somatic Cell Count, and Milk Composition

The presence of *M. bovis* DNA in milk samples was associated with a lower (−3.0 kg) daily milk yield compared with dairy cows without *M. bovis* DNA in the milk samples. The daily milk yield was also lower (−4.0 kg) in dairy cows that tested positive for *Strep. agalactiae* compared with *Strep. agalactiae* negative dairy cows (Table 3).

The lnSCC was significantly higher in milk samples, in which DNA from *M. bovis*, *Staph. aureus*, and *Strep. agalactiae* was detected, compared with milk samples negative for these pathogens (Table 4).

In the CMS of cows positive for *M. bovis*, the milk fat and urea ($P < 0.05$) content were lower compared with the respective values of cows negative for *M. bovis*. *Mycoplasma bovis* was not significantly associated with milk protein content (Tables 5, 6, and 7). The presence of *Strep. agalactiae* in milk samples had a positive association with the milk fat content ($P = 0.041$) and protein content ($P = 0.034$; Tables 5 and 6).

DISCUSSION

Prevalence of Mycoplasma bovis in Cow CMS Using qPCR

The within-herd prevalence of subclinical *M. bovis* udder infection is not widely studied in European dairy herds. Most of the previous studies are focusing on clinical mastitis cases or on between-herd prevalence of *M. bovis* (Filioussis et al., 2007; Arcangioli et al., 2011; Radaelli et al., 2011; Passchyn et al., 2012). We identified a *M. bovis* within-herd prevalence of 17.2% in one dairy herd. Previously, Brown et al. (1990) identified a 5.7% (n = 1535) prevalence of mycoplasmas based on a study in one herd. According to the study of Brown et al. (1990), a higher within-herd prevalence of *M. bovis*

Table 3. Results of multivariable linear regression model of association between detected mastitis pathogens in cow composite milk samples and daily milk yield (kg) of dairy cows (n = 520)

Item	Number of cows	Coefficient	95% CI	*P*-value	Wald test
Mycoplasma bovis negative	430	0			
Mycoplasma bovis positive	90	−3.0	−5.2; −0.8	0.007	
Staphylococcus aureus negative	467	0			
Staphylococcus aureus positive	53	0.8	−1.8; 3.5	0.546	
Streptococcus agalactiae negative	372	0			
Streptococcus agalactiae positive	148	−4.0	−5.9; −2.2	<0.001	
1 Lactation	220	0			<0.001
2 Lactations	159	3.7	1.9; 5.5	<0.001	
≥3 Lactations	141	3.3	1.4; 5.3	0.001	
<90 DIM	133	0			<0.001
91–200 DIM	170	−4.4	−6.5; −2.3	<0.001	
≥201 DIM	217	−13.6	−15.6; −11.6	<0.001	
Intercept		36.0	34.2; 37.9	<0.001	

Table 4. Results of multivariable linear regression model of association between SCC in cow composite milk samples and mastitis pathogens (n = 520)

Item	Number of cows	Coefficient[1]	95% CI	*P*-value	Wald test
Mycoplasma bovis negative	430	0			
Mycoplasma bovis positive	90	0.8	0.5; 1.1	<0.001	
Staphylococcus aureus negative	467	0			
Staphylococcus aureus positive	53	0.9	0.5; 1.3	<0.001	
Streptococcus agalactiae negative	372	0			
Streptococcus agalactiae positive	148	0.6	0.3; 0.8	<0.001	
1 Lactation	220	0			<0.001
2 Lactations	159	0.1	−0.2; 0.4	0.459	
≥3 Lactations	141	0.6	0.3; 0.8	<0.001	
<90 DIM	133	0			<0.001
91–200 DIM	170	0.1	−0.2; 0.4	0.399	
≥201 DIM	217	0.5	0.2; 0.8	<0.001	
Intercept		3.7	3.4; 4.0	<0.001	

[1]Estimates are in logarithmic scale.

Table 5. Results of multivariable linear regression model of association between milk fat in cow composite milk samples and mastitis pathogens (n = 520)

Item	Number of cows	Coefficient[1]	95% CI	*P*-value	Wald test
Mycoplasma bovis negative	430	0			
Mycoplasma bovis positive	90	−0.05	−0.1; −0.004	0.035	
Staphylococcus aureus negative	467	0			
Staphylococcus aureus positive	53	−0.1	−0.07; 0.05	0.732	
Streptococcus agalactiae negative	372	0			
Streptococcus agalactiae positive	148	0.05	0.002; 0.09	0.041	
1 Lactation	220	0			0.021
2 Lactations	159	−0.06	−0.1; −0.01	0.011	
≥3 Lactations	141	−0.05	−0.1; −0.001	0.046	
<90 DIM	133	0			<0.001
91–200 DIM	170	−0.03	−0.08; 0.02	0.233	
≥201 DIM	217	0.08	0.04; 0.1	0.001	
Intercept		2.0	1.95; 2.04	<0.001	

[1]Estimates are on a square root scale.

Table 6. Results of multivariable linear regression model of association between milk protein in cow composite milk samples and mastitis pathogens (n = 520)

Item	Number of cows	Coefficient[1]	95% CI	*P*-value	Wald test
Mycoplasma bovis negative	430	0			
M. bovis positive	90	0.001	−0.005; 0.007	0.706	
Staphylococcus aureus negative	467	0			
Staph. aureus positive	53	0.002	−0.006; 0.009	0.674	
Streptococcus agalactiae negative	372	0			
Strep. agalactiae positive	148	−0.006	−0.01; −0.0004	0.034	
1 Lactation	220	0			0.282
2 Lactations	159	0.003	−0.002; 0.008	0.214	
≥3 Lactations	141	0.004	−0.002; 0.009	0.190	
<90 DIM	133	0			<0.001
91–200 DIM	170	−0.02	−0.02; −0.01	<0.001	
≥201 DIM	217	−0.04	−0.05; −0.04	<0.001	
Intercept		0.3	0.30; 0.31	<0.001	

[1]Estimates are on an inverse scale (negative estimate means higher content of protein).

IMI was found in our study. However, we collected milk samples only once and from a single dairy herd by using a high-sensitivity qPCR method to detect major udder pathogens from the CMS (Koskinen et al., 2010; Murai et al., 2014; Nyman et al., 2016). Therefore, the results of this study describe the within-herd prevalence of *M. bovis* only at the single point in time in one specific herd.

Cow CMS are used to detect especially subclinical IMI when it is difficult to identify the infected udder quarter. However, milk originating from noninfected udder quarters may lower the sensitivity in IMI pathogen detection, due to a dilution effect (Reyher and Dohoo, 2011). In this study, we used commercial qPCR analysis to detect 4 mastitis pathogens in cow CMS simultaneously. A high sensitivity of the PCR method to detect udder pathogens causing IMI is reported, also when cow CMS are used (Friendship et al., 2010; Koskinen et al., 2010; Murai et al., 2014; Nyman et al., 2016).

A carry-over of the udder pathogen DNA may occur when CMS are collected with automated milk meters. Milk from a previously milked cow is mixed with milk from the cow currently being milked, leading to false-positive PCR test results (Løvendahl et al., 2010; Mahmmod, 2015). In conventional milking systems, the probability of carry-over is evaluated to range from 2 to 3.5% (Løvendahl and Bjerring, 2006; Løvendahl et al., 2010). The carry-over effect may be associated with the Ct-value cut-off (Mahmmod et al., 2014). Lower cut-off values in discriminating positive and negative test results lower the sensitivity of the test, but reduce the number of false-positive test results that may occur due to carry-over (Mahmmod et al., 2014). The cut-off value was set to be ≤37.0 in our study, which should be low enough to rule out most of the false-positive results and hence decrease the probability that contaminated samples are classified as positive (Mahmmod et al., 2014).

Associations Between Cow Mycoplasma bovis Infection and Milk Yield and Milk Composition

To our knowledge this is the first study evaluating associations between subclinical *M. bovis* IMI, cow milk yield, SCC, and composition, when cow CMS are used. We identified no significant relation between co-infection with udder pathogens and cow milk yield, SCC, or milk composition. This may be due to relatively low numbers of samples with multiple pathogens detected and should be controlled in further studies using larger number of samples.

The presence of *M. bovis* bacterial DNA in CMS was associated with an average of a 3.0 kg lower daily milk yield compared with cows negative for *M. bovis.* The negative effect of *Strep. agalactiae* or *Staph. aureus* subclinical IMI on milk yield was already discovered decades ago (Keefe et al., 1997; Reksen et al., 2007). In the present study, a negative association between presence of bacterium in subclinically infected cow CMS and milk yield was detected for *Strep. agalactiae* but not for *Staph. aureus.* Even though the number of *M. bovis* positive dairy cows was low in our study, a significant relation between *M. bovis* and cow daily milk yield was identified. As far as we know, no research has been published about the association between subclinical *M. bovis* IMI and milk yield. Therefore, this study provides essential knowledge about the economic effect of *M. bovis* on dairy farm production.

A significant association was observed between *M. bovis* and milk SCC level after controlling for the presence of other mastitis pathogens, parity, and DIM. We found that in CMS positive for *M. bovis*, the lnSCC was on average 0.8 units higher compared with milk

Table 7. Results of multivariable linear regression model of association between milk urea (mg/L) in cow composite milk samples and mastitis pathogens (n = 520)

Item	Number of cows	Coefficient	95% CI	*P*-value	Wald test
Mycoplasma bovis negative	430	0			
M. bovis positive	90	−15.6	−25.6; −5.6	0.002	
Staphylococcus aureus negative	467	0			
Staph. aureus positive	53	−6.0	−18.2; 6.3	0.339	
Streptococcus agalactiae negative	372	0			
Strep. agalactiae positive	148	−7.8	−16.4; 0.7	0.072	
1 Lactation	220	0			<0.001
2 Lactations	159	−3.8	−12.2; 4.7	0.379	
≥3 Lactations	141	−16.8	−25.7; −7.9	<0.001	
<90 DIM	133	0			<0.001
91–200 DIM	170	28.2	18.7; 37.7	<0.001	
≥201 DIM	217	17.1	8.1; 26.2	<0.001	
Intercept		159.4	150.8; 168.0	<0.001	

samples negative for this pathogen. It is speculated that the SCC of cows positive for *M. bovis* is higher in most of the cows, but not all of them (Ghadersohi et al., 1999; Pinho et al., 2013). Even though in our study there was an association between *M. bovis* IMI and cow SCC, using only cow SCC status in mastitis control programs may lead to an omission of some *M. bovis* positive dairy cows (Ghadersohi et al., 1999; Pinho et al., 2013).

In our study, the subclinical *M. bovis* udder infection was negatively associated with some of the milk composition parameters. The milk fat and urea content in *M. bovis*-positive cows was lower than in *M. bovis*-negative cows. Spontaneous lipolysis during the IMI leads to lower milk fat content and changes in milk fat composition (Forsbäck et al., 2010). Urea is formed in the liver from ammonium and absorbed to the milk from the blood in a stable form (Hayton et al., 2012). However, many factors influence the milk urea content, such as altered DMI, or the lack of rumen degradable protein and energy in feed ratio (Rezamand et al., 2007; Hayton et al., 2012). Therefore, it is not possible to make strong conclusions about the causality of *M. bovis* subclinical IMI and milk urea content.

Intramammary infection usually causes a decrease in milk protein levels (Rezamand et al., 2007). In this study, we did not find a significant association between the presence of *M. bovis* in milk samples and milk protein content. Further studies using a larger sample size are needed to confirm the associations between subclinical *M. bovis* IMI and milk yield as well as milk composition parameters, making it possible to estimate the economic effect of *M. bovis* IMI and effects on cow well-being.

CONCLUSIONS

This study identified the within-herd prevalence of subclinical *Mycoplasma bovis* IMI of 17.2% by testing CMS of 522 lactating dairy cows using qPCR. Dairy cows infected with *M. bovis* had higher SCC, produced less milk, and had lower milk fat and urea content compared with *M. bovis*-negative dairy cows. Further studies should evaluate the associations between *M. bovis* and milk yield and milk quality in a larger number of herds. Our findings underlay the importance to apply control measures of *M. bovis* mastitis to reduce economic losses due to lower milk yield and milk quality caused by subclinical udder infection of *M. bovis*.

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